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REVIEW

mTOR in Lung Neoplasms

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Abstract
With the discovery of rapamycin 45 years ago, studies in the mechanistic target of rapamycin (mTOR) field started 2 decades before the identification of the mTOR kinase. Over the years, studies revealed that the mTOR signaling is a master regulator of homeostasis and integrates a variety of environmental signals to regulate cell growth, proliferation, and metabolism. Deregulation of mTOR signaling, particularly hyperactivation, frequently occurs in human tumors. Recent advances in molecular profiling have identified mutations or amplification of certain genes coding proteins involved in the mTOR pathway (eg, *PIK3CA*, *PTEN*, *STK11*, and *RICTOR*) as the most common reasons contributing to mTOR hyperactivation. These genetic alterations of the mTOR pathway are frequently observed in lung neoplasms and may serve as a target for personalized therapy. mTOR inhibitor monotherapy has met limited clinical success so far; however, rational drug combinations are promising to improve efficacy and overcome acquired resistance. A better understanding of mTOR signaling may have the potential to help translation of mTOR pathway inhibitors into the clinical setting.

Keywords Lung neoplasms · mTORC1 · mTORC2 · mTOR inhibitors · mTOR signaling

Introduction

Rapamycin was discovered as an antifungal medication 45 years ago, when Sehgal and colleagues were looking for new antimicrobial agents on Easter Island [1]. Shortly thereafter, it was identified that rapamycin also has immunosuppressive and antitumor effects [2, 3]. The mechanism of action, however, remained elusive for another 20 years. Twenty-five years ago, Sabatini et al and Sabers et al described that the mechanistic (formerly *mammalian*) target of rapamycin (mTOR) is a protein kinase (mTOR kinase) and it is the direct target of the rapamycin-FK506 binding protein 12 (FKBP12) complex [4, 5]. Over the last few decades, studies from different research groups have tried to solve the big puzzle of this cellular signaling network with the mTOR kinase in its centerpiece. It has been revealed that the mTOR pathway has a fundamental role in integrating environmental signals and responding to them adequately [6]. In this short review, we provide an overview of the regulatory disturbances of mTOR and recent therapeutic interventions regarding the mTOR pathway in lung neoplasms.

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The mTOR Kinase

mTOR is a serine/threonine protein kinase that forms the catalytic subunit of 2 structurally and functionally distinct multiprotein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [7, 8]. The mTOR complexes differ in their rapamycin sensitivity, protein components, subcellular localization, upstream regulation, and downstream effectors [9, 10]. Through these complexes, the mTOR pathway integrates diverse environmental and nutritional signals to regulate essential cellular functions, such as survival, cell growth, and proliferation. The mTOR kinase is an integral part of the phosphatase and tensin homolog (PTEN)/ phosphatidylinositol-3-kinase (PI3K)/ protein kinase B (Akt) axis, moreover, it is interconnected with many other crucial pathways (eg, the rat sarcoma viral oncogene homolog [Ras]/rapidly accelerated fibrosarcoma [Raf]/mitogen-activated protein kinase kinase [MEK]/extracellular signal-regulated kinase [ERK] pathway), establishing a central junction in a network of signaling cascades [7, 11].

mTOR Complex 1

mTORC1 is composed of mTOR, regulatory-associated protein of mTOR (Raptor), mammalian lethal with SEC13 protein 8 (mLST8), and the 2 inhibitory subunits DEP domain-containing mTOR-interacting protein (DEPTOR) and proline-rich Akt substrate of 40 kDa [12]. While suppressing certain catabolic pathways, cells must increase the availability of proteins, lipids, and nucleotides via anabolic processes to allow growth and proliferation. mTORC1 has a central role in the regulation of these metabolic processes, thereby controlling the balance between anabolism and catabolism that permits adaptation to changing environmental conditions [8].

Oncogenic activation of mTORC1 can happen through the PI3K/Akt pathway in response to aberrant activation of receptor tyrosine kinases (RTKs) or genetic alterations involving the PI3K/Akt/mTOR pathway [10, 11]. On the other hand, high energy state and oxygen also stimulates mTORC1 activity through blocking 5′-AMP-activated protein kinase (AMPK) that works as an energy sensor and a negative regulator of the mTOR signaling [13]. Amino acids, particularly leucine and arginine, also activate mTORC1 via stimulation of the Rag GTPases that promote lysosomal localization of mTORC1, thereby enabling it to encounter its activator, Ras homolog enriched in brain (Rheb) [14, 15].

Once activated, mTORC1 transduces the signaling to downstream effectors regardless of its activation source. Activated mTORC1 phosphorylates its main downstream effectors eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and ribosomal protein S6 kinase 1 (S6K1), both of which are essential regulators of protein translation. Activation of 4EBP1 and S6K1 results in an increase in cell size and proliferation, which are typical characteristics of cancer [10, 16]. In order to fulfill the biosynthetic needs of proliferating cells, mTORC1 also acts on other molecules such as Lipin1 [17], sterol regulatory element binding proteins [18], carbamoyl-phosphate synthetase 2, aspartate transcarbamoylase, and dihydroorotase [19], and activating transcription factor 4 [20] to promote the production of lipids and nucleotides. In addition to its anabolic effects, mTORC1 also supports cell growth by promoting protein catabolism. Most notably, it regulates autophagy and lysosome biogenesis via phosphorylation of the Unc-51-like kinase 1 [21] and transcription factor EB [22], and encourages the activity of the ubiquitin-proteasome system. Furthermore, mTORC1 also regulates mitochondrial biogenesis via peroxisome proliferator-activated receptor gamma coactivator 1α [23] and under hypoxic conditions, it supports the metabolic shift from oxidative phosphorylation to glycolysis through the increased translation of hypoxia inducible factor 1α (HIF1α), which promotes the expression of several glycolytic enzymes [24–26]. Cellular reactive oxygen species (ROS) levels are also highly dependent on mTORC1, which regulates superoxide dismutase 1 activity and, therefore, is able to minimize oxidative damages under nutrient stress [27].

mTOR Complex 2

In addition to the catalytic subunit mTOR kinase, mTORC1 and mTORC2 also share the core component mLST8 and the inhibitory subunit DEPTOR. Instead of Raptor, however, mTORC2 contains the scaffold protein rapamycin-insensitive companion of mTOR (Rictor). In addition to these components, mTORC2 contains the regulatory subunits mammalian stress-activated protein kinase-interacting protein 1 and protein observed with Rictor 1/2 (Protor1/2) [28]. In contrast to mTORC1, mTORC2 is not bound by the rapamycin-FKBP12 complex, therefore, it is considered to be resistant to short-term rapamycin treatment. However, it has been described that prolonged rapamycin treatment can abrogate mTORC2 signaling, likely due to inhibition of the assembly of mTORC2 [8, 29].

The upstream regulation of mTORC2 remains less well defined than that of mTORC1. Growth factors can activate mTORC2 via PI3K at the plasma membrane, moreover, under energetic stress conditions, it can be activated in an AMPK-dependent manner [30]. In addition, protein components of mTORC2 are subjected to posttranslational modifications, including phosphorylation, acetylation, and ubiquitination, which may have an important role in the assembly and activation of mTORC2 [31]. Even the scaffold protein of mTORC2, Rictor, contains multiple modifiable sites, which might have an impact on mTORC2 activity. Rictor can be phosphorylated at Thr1135 by S6K1 and at Ser1235 or Thr1695 by glycogen synthase kinase 3 (GSK3), but the effect of these modifications on the mTORC2 activity has not been fully elucidated [32–34].

While mTORC1 primarily controls cell growth and metabolism, mTORC2 instead regulates proliferation, survival, actin cytoskeleton reorganization and, therefore, cell migration. Besides phosphorylation of protein kinase C (PKC) and serum glucocorticoid-regulated kinase 1 (SGK1), activation of mTORC2 leads to phosphorylation of Akt at Ser473, which, in turn, can positively regulate mTORC1 activity [7, 8, 29].

Regulation of mTOR Signaling

mTORC1 integrates several intra- and extracellular signals —such as growth factors, DNA damage, energy status, oxygen, and amino acid availability —to regulate fundamental processes that are involved in promotion of cell growth and proliferation [6, 8]. mTORC2 also responds to growth factors in a poorly defined, PI3K-dependent fashion [35]. It has been recently described that AMPK can activate mTORC2 [30];

however, in contrast to mTORC1, mTORC2 is generally considered to be insensitive to nutrients [6].

The PI3K/Akt/mTOR signaling pathway can be primarily activated through RTKs (eg, epidermal growth factor receptor, insulin-like growth factor receptor 1, vascular endothelial growth factor receptor, and platelet-derived growth factor receptor) and is involved in several biologic functions such as proliferation, differentiation, survival, adhesion, motility, invasion, and cellular metabolism [8, 36]. At the plasma membrane, activated RTKs recruit a complex containing PI3K that phosphorylates the phosphatidylinositol lipid substrates, resulting in the production of phosphatidylinositol (3,4,5)-trisphosphate (PIP_3). PIP_3 transduces the signaling from the membrane to the cytoplasm through multiple effector proteins [36, 37]. The tumor suppressor PTEN counteracts the PI3K signaling by dephosphorylating PIP_3 to phosphatidylinositol (4,5)-bisphosphate (PIP_2) [38]. Production of PIP_3 induces the recruitment of Akt to the plasma membrane and causes its activation by phosphorylation at Thr308. The Akt-dependent phosphorylation of tuberous sclerosis complex 2 (TSC2), a critical negative regulator of mTORC1, then subsequently activates Rheb and mTORC1, thereby stimulating protein synthesis, cell growth, and proliferation (Fig. 1) [39].

The serine/threonine kinase Akt exists in 3 different isoforms: Akt1, Akt2, and Akt3. Akt1 and Akt2 are widely expressed in multiple tissues. In contrast, Akt3 is predominantly expressed in the brain, kidney, and heart [37]. The pleckstrin homology domains of both phosphoinositide-dependent kinase-1 (PDK1) and Akt can bind to PIP_3, and this colocalization allows PDK1 to access and phosphorylate Akt at Thr308, leading to partial activation. mTORC2 can also phosphorylate Akt at the hydrophobic motif Ser473, which further increases enzymatic activity [40, 41]. In addition to its indirect activating effect on mTORC1, Akt regulates several downstream signaling proteins by phosphorylation, such as GSK3, forkhead box O transcription factors, and B-cell lymphoma 2-associated agonist of cell death, thereby stimulating cell cycle progression and promoting survival, while suppressing apoptotic signals [36].

In addition to the activating effect of growth factors and insulin on the PI3K/Akt/mTOR pathway, different nutritional and environmental signals such as high adenosine triphosphate (ATP) levels, oxygen, and increased serum amino acid levels can also increase the activity of mTORC1. In contrast, intracellular and environmental stress signals such as low ATP levels, hypoxia, and DNA damage inhibit the activity of mTORC1, mainly through the activation of AMPK [8].

In addition to mTORC1, AMPK is also defined as a master regulator of cellular metabolism and has a fundamental role in nutrient and glucose sensing [13]. In response to energy depletion or hypoxia, activated AMPK phosphorylate TSC2 and Raptor, thereby leading to the inhibition of mTORC1. The tumor suppressor liver kinase B1 (Lkb1, encoded by the *STK11* gene) acts as a negative regulator of mTORC1 by phosphorylation and activation of AMPK under certain environmental conditions [15, 37, 39].

Under hypoxia, the reduction in ATP levels can result in activation of AMPK and subsequent inhibition of mTORC1 [42]. Moreover, low oxygen level results in an increase in the expression of HIF1α, which becomes stabile and active as a transcriptional factor together with hypoxia inducible factor 1β (HIF1β) and regulates the expression of several target genes involved in glycolysis (glucose transporter 1), angiogenesis (vascular endothelial growth factor A), and pH regulation (carbonic anhydrase IX) [43]. Hypoxia also induces the expression of transcriptional regulation of DNA damage response 1, which activates TSC2 function and, therefore, inhibits mTORC1 [44].

The presence of amino acids is essential for mTORC1 activation. Under amino acid starvation, mTORC1 cannot be fully activated even in TSC knockout cells [45, 46]. In contrast, elevated amino acid levels can positively regulate mTORC1 mainly through the activation of Rag GTPases by the cytosolic amino acid sensors Sestrins, cellular arginine sensor for mTORC1 (CASTOR) and S-adenosylmethionine sensor upstream of mTOR (SAMTOR) [14, 45]. Rag GTPases are tethered to the lysosomal membrane [47] and in the presence of amino acids, cells switch them to their active heterodimeric conformation allowing them to bind Raptor, thereby recruiting mTORC1 to the lysosomal surface that also contains its activator, Rheb [48].

Alterations of the mTOR Pathway in Lung Neoplasms

Dysregulation of the mTOR signaling pathway is implicated in the pathogenesis of many human cancers, including lung neoplasms [10]. Aberrant activation of the mTOR pathway can occur through a variety of mechanisms, including genetic alterations involving *PIK3CA*, *PTEN*, *STK11*, *AKT*, *TSC1*, *TSC2*, *RICTOR*, *MTOR*, and other related oncogenes or tumor suppressor genes [49, 50]. Additionally, mTOR activation in lung cancer can also be affected by diverse genetic alterations of many associated signaling pathways, including mutations causing constitutive activation of epidermal growth factor receptor (EGFR) and Kirsten rat sarcoma viral oncogene homolog (KRAS) [51, 52]. These alterations offer therapeutic opportunities to target the PI3K/AKT/mTOR pathway in cancer.

Lung Adenocarcinomas and Squamous Cell Carcinomas

Aberrant activation of the PI3K/Akt/mTOR pathway has been found in 90% of lung adenocarcinomas (ADCs) and 40% of squamous cell carcinomas (SCC) [53]. Deregulated mTOR

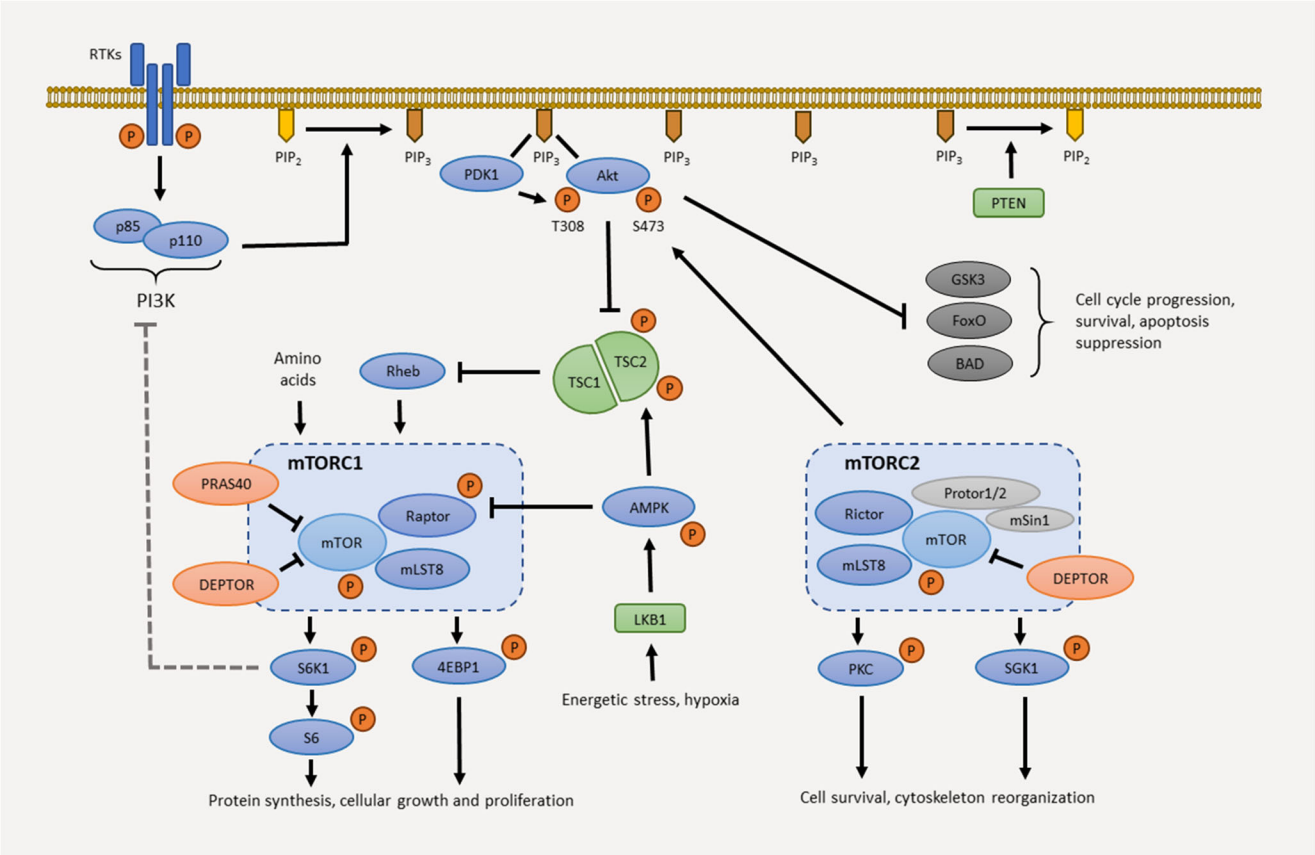

Fig. 1 Simplified Scheme of the Mechanistic Target of Rapamycin (mTOR) Signaling Pathway. The phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt)/mTOR pathway can be activated by growth factor receptor tyrosine kinases, which recruit PI3K proteins to the plasma membrane. The class I PI3K proteins phosphorylate phosphatidylinositol (4,5)-bisphosphate (PIP_2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) that acts as a second messenger and activates Akt via phosphorylation on T308. The phosphatase and tensin homolog (PTEN) can dephosphorylate PIP_3 to PIP_2, thereby counteracts the effect of PI3K and inhibits the activity of the signaling pathway. The activated Akt phosphorylates tuberous sclerosis complex 2 (TSC2) leading to the dissociation and inactivation of the TSC1/2 complex. The loss of the inhibitory effect of the TSC1/2 complex activates mTOR complex 1 (mTORC1) and leads to phosphorylation of its main downstream effectors, ribosomal protein S6 kinase 1 (S6K1) and factor 4E-binding protein 1 (4E-BP1), thereby increases protein synthesis and supports cell growth and proliferation. mTORC1 is also implicated in a negative feedback loop (dashed grey line) via S6K1 that can prevent Akt overactivation. The factors that activate mTOR complex 2 (mTORC2) are less well elucidated. Once active, mTORC2 phosphorylates its downstream targets, serum glucocorticoid-regulated kinase 1 (SGK1) and protein kinase C (PKC), hence promotes cell survival and regulates the remodeling of the actin cytoskeleton and cell migration. mTORC2 can also phosphorylate Akt on S473 leading to its full activation. In addition to its activating effect on mTORC1, Akt can promote cell cycle progression, survival and suppress apoptosis through glycogen synthase kinase 3 (GSK3), forkhead box O (FoxO), and BCL2-associated agonist of cell death (BAD), as well. Under energetic stress and hypoxia, the tumor suppressor liver kinase B1 (LKB1) also decreases the activity of mTORC1 via phosphorylation of TSC2 and regulatory-associated protein of mTOR (Raptor) by 5′-AMP-activated protein kinase (AMPK), which acts as an energy sensor of the cell. DEPTOR indicates domain-containing mTOR-interacting protein; mLST8, mammalian lethal with SEC13 protein 8; mSin1, mammalian stress-activated map kinase-interacting protein 1; P, phospho; PDK1, phosphoinositide-dependent kinase-1; PRAS40, proline-rich Akt substrate of 40 kDa; p85, regulatory subunit of PI3K; p110, catalytic subunit of PI3K; Proctor1/2, protein observed with Rictor 1/2; Rheb, Ras homolog enriched in brain; Rictor, rapamycin-insensitive companion of mTOR; RTK, receptor tyrosine kinase; S6, ribosomal protein S6

activity is known to contribute to lung cancer development and maintenance, and its activated downstream effectors, phospho-eukaryotic translation initiation factor 4E and phospho-S6 ribosomal protein (p-S6), are overexpressed in both ADCs and SCCs. Moreover, activation of the mTOR pathway has been found to be associated with unfavorable clinical outcome, invasiveness, and metastasis formation [54–57].

Dysregulation of the PI3K/Akt/mTOR pathway occurs through multiple mechanisms including activation of RTKs upstream of PI3K, activating genetic alterations in the *PIK3CA*, *AKT,* and *RICTOR* genes, or loss of the tumor suppressor genes *PTEN* or *STK11* (Table 1) [7, 37, 58, 59]. *PIK3CA* activating mutations have been reported with a frequency of 5% and 7% in ADCs and SCCs, respectively, whereas *PIK3CA* amplification has been found in about 33% of SCCs versus 1% of ADCs. Amplification of the *RICTOR* gene has been observed in about 10% of the ADC and SCC cases. The tumor suppressor genes *PTEN* and *STK11*

act as negative regulators of the mTOR signaling and have also been found to be frequently mutated in lung cancer. Loss of *PTEN* is a common finding in SCC, whereas loss-of-function mutation of *STK11* has been frequently observed in ADCs [58–60].

KRAS mutations are found in about 25% to 35% of ADCs resulting in the highest mutation rate among all lung cancer subtypes. Other molecular alterations related to Ras pathway activation are present in about 25% of the ADC cases, such as *EGFR* and *BRAF* mutations [61]. Activated KRAS promotes oncogenic transformation by increasing the activity of the downstream signaling cascades, including Raf/MEK/ERK and PI3K/Akt/mTOR pathway. Inhibition of these pathways appears to be a rational approach to treatment, however, agents targeting PI3K/Akt/mTOR signaling have not been shown to be effective against RAS-driven cancers as single agents [62].

Pulmonary Neuroendocrine Tumors

Lung neuroendocrine tumors (LNETs) are classified into 4 histological variants (typical carcinoid, atypical carcinoid, small cell lung carcinoma [SCLC] and large cell neuroendocrine carcinoma) and these subtypes share many similarities in terms of histologic structure, immunohistochemical features, and molecular biology [63–66]. Low/intermediate-grade carcinoids and high-grade carcinomas harbor mostly the same genetic alterations but with different prevalence rates. In contrast to carcinoids, aberrations in the genes involved in the PI3K/Akt/mTOR pathway are significantly enriched in carcinomas. Additionally, there are more copy number alterations in carcinomas than in carcinoids. *RICTOR*, encoding a component of mTORC2, is 1 of the most frequently amplified genes in LNETs with a significant enrichment in carcinomas as compared to carcinoids [66].

As the most common subtype of LNETs, SCLC accounts for approximately 15% to 20% of all newly diagnosed lung cancers [67]. In addition to mutations in the *TP53* and *RB1* cell cycle regulation genes and amplification of *MYC* family members, genetic alterations in the PI3K/Akt/mTOR pathway (eg, *PIK3CA*, *PTEN*, *AKT2*, *AKT3*, *MTOR*, and *RICTOR*) have been frequently observed in SCLC (Table 1) [68–70]. Among the most frequently altered genes, *RICTOR* amplification (Fig. 2a) is the most common targetable gene alteration in SCLC that has been found in 6% to 15% of cases [71–73].

Activation of the mTOR pathway has also been identified using immunohistochemistry in SCLC (Fig. 2b). Positive staining for p-mTOR (the active form of mTOR kinase), p-S6K (a downstream target of mTORC1), Rictor (a scaffold protein of mTORC2) and p-Akt (a downstream target of mTORC2) has been found in 55%, 84%, 37%, and 42% of patients, respectively [71, 74]. Moreover, high expression of both Rictor and p-Akt have been associated with metastatic disease and decreased survival [71].

Lymphangioleiomyomatosis

Lymphangioleiomyomatosis (LAM) is a rare cystic lung disease that is considered as a low-grade neoplasm of the perivascular epithelioid cell tumor family [75]. Proliferation of smooth muscle-like LAM cells causing cystic lung destruction is the main feature of LAM. It can occur sporadically (S-LAM) or in association with the heritable disease, tuberous sclerosis complex (TSC-LAM). In both TSC-LAM and S-LAM, loss-of-function mutations in the *TSC* genes result in constitutive activation of the mTOR pathway, leading to proliferation, growth, invasion, and migration of LAM cells and destructive tissue remodeling [76–78].

In addition to mutation analyses of the *TSC1* and *TSC2* genes [79–81], the presence of high mTORC1 activity has also been proven in LAM by immunohistochemical analysis of its downstream targets p-S6K, p-S6 and p-4E-BP1 [79, 82–84]. Moreover, the importance the mTORC2 activity in the pathobiology of LAM has also emerged. Rictor overexpression has been found in 55% of the cases suggesting that dual mTORC1/2 inhibitors may be worthy of clinical investigation for the treatment of LAM [84].

Inhibitors of mTOR Signaling in Clinical Development

Therapeutic investigations with PI3K/Akt/mTOR pathway inhibitors have resulted in the development of more than 40 different drugs. Although, several of them were tested in various stages of clinical trials, only a few —including the allosteric mTORC1 inhibitors temsirolimus and everolimus, and the PI3K inhibitors idelalisib and copanlisib —have been approved for the treatment of cancer [50]. In addition, the mTORC1 inhibitor sirolimus has been approved for the treatment of LAM [85].

Most inhibitors of the mTOR pathway are associated with a limited single-agent activity. In case of mTORC1 inhibitors, this phenomenon can be explained by disruption of the mTORC1/S6K1-mediated negative feedback loop, which paradoxically results in activation of Akt through PI3K and mTORC2 signaling (Fig. 1) [29]. Early clinical data suggest that combinations of the PI3K/Akt/mTOR pathway inhibitors with chemotherapy or different targeted agents are more effective than monotherapy alone. Combination strategies might be useful to increase efficacy and overcome intrinsic and acquired resistance in the treatment of cancer; however, biomarker-based patient selection and toxicity issues are of paramount importance for clinical success [50, 86].

Table 1 Alterations of the PI3K/Akt/mTOR pathway in selected lung neoplasms

Gene	Protein	Alteration type	Alteration frequency (%)			References for SCLC data
			ADC*	SCC*	SCLC	
PIK3CA	PI3K p110α	Mutation	5	7	3-6	[68–70, 72]
		Copy number gain	1	33	2-3	
PTEN	Pten	Mutation	2	21	2-6	[68–70, 72]
STK11	Lkb1	Mutation	15	2	<1	[68, 69]
AKT1	Akt1	Mutation	<1	1	2	[68, 69]
		Copy number gain	<1	3	<1	
AKT2	Akt2	Mutation	1	1	1-4	[68–70]
		Copy number gain	1	6	9	
AKT3	Akt3	Mutation	2	<1	2-4	[68–70]
		Copy number gain	5	3	<1	
TSC1	Hamartin	Mutation	2	2	0-3	[68, 69, 72]
TSC2	Tuberin	Mutation	3	3	2	[68, 69]
MTOR	mTOR	Mutation	5	4	2-8	[68–70]
		Copy number gain	<1	<1	<1	
RICTOR	Rictor	Mutation	3	3	2-3	[68–73]
		Copy number gain	8	10	6-15	

ADC, adenocarcinoma; *Akt*, protein kinase B; *mTOR*, mechanistic target of rapamycin; *PI3K*, phosphatidylinositol-3-kinase; *SCC*, squamous cell lung carcinoma; *SCLC*, small cell lung carcinoma

*TCGA data was accessed using the cBioPortal (http://www.cbioportal.org/, TCGA PanCancer Atlas)

mTORC1 Inhibitors

Allosteric inhibitors of the mTORC1 include rapamycin (also known as sirolimus), everolimus, temsirolimus, and ridaforolimus, and represent the most developed class of PI3K/Akt/mTOR pathway inhibitors [87].

The inhibitors of mTORC1 have been extensively studied in multiple clinical trials for the treatment of lung ADCs and SCCs. Because of the modest single-agent activity [88], trials have focused on finding effective combinations. Phase I/II combination studies are ongoing to study the efficacy of mTORC1 inhibitors in combination with other agents in non-small cell lung carcinoma (NSCLC) (Table 2).

The RADIANT studies have demonstrated that everolimus provide clinically meaningful improvement in progression-free survival in patients with low- or intermediate-grade LNETs [89–91]. In the LUNA phase II study, everolimus treatment in combination with long-acting pasireotide has also showed an acceptable safety profile and preliminary evidence of antitumor activity in patients with advanced carcinoid tumors of the lung [92]. Intravenously administered nanoparticle albuminbound (nab)rapamycin (ABI009), which has an improved bioavailability as compared to oral rapamycin, is currently being evaluated in phase II studies in LNETs (Table 2). In SCLC, everolimus and temsirolimus have been tested, however, limited single-agent antitumor activity has been observed in unselected patients [93, 94].

The safety and efficacy of sirolimus and everolimus in LAM have been tested in a number of clinical studies [95–98]. Primarily based on findings from the MILES study [97], sirolimus has been approved for the treatment of LAM [85, 99]. Currently, 2 ongoing clinical trials are investigating the efficacy of low-dose sirolimus treatment and combination of sirolimus with resveratrol in LAM patients (Table 2) [100, 101].

mTORC1/2 Inhibitors

ATP-competitive (catalytic) inhibitors of mTOR kinase effectively target both mTORC1 and mTORC2, resulting in a higher level of inhibition of the mTOR pathway and, hence, improved anticancer activity. Additionally, in contrast to allosteric mTOR inhibitors, mTOR kinase inhibitors also have the potential to prevent the feedback loop-based activation of Akt [29, 50].

Vistusertib is under investigation in multiple phase I and II trials involving patients with lung cancer. In some phase II studies, patients are selected based on genetic alterations which result in hyperactivation of mTORC1 and/or mTORC2 and, therefore, are hypothesized to have an increased sensitivity to the drug. Vistusertib has been reported to be highly active against SCC in combination with paclitaxel in a phase I study [102] and is also under investigation in rationally designed combinations with other therapies, such as selumetinib, navitoclax, and durvalumab (Table 2) in order to overcome intrinsic and acquired resistance mechanisms and improve patient outcomes.

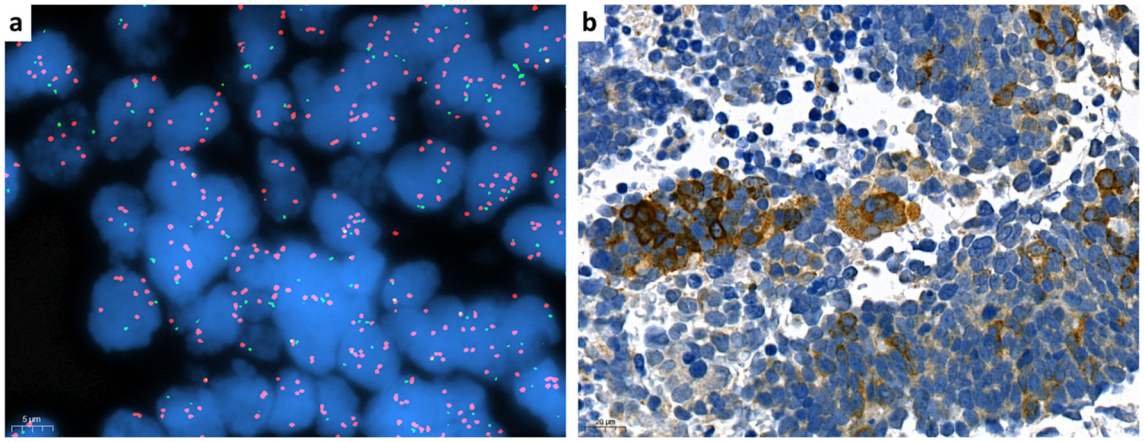

Fig. 2 Rapamycin-insensitive Companion of mTOR (*RICTOR*) Fluorescence in situ Hybridization (FISH) and Rictor Immunohistochemistry in Small Cell Lung Carcinoma (SCLC). *RICTOR* amplification was detected by FISH (**a**) in a cell block from a lymph node metastasis of an SCLC (original magnification, x1000). Rictor expression was analyzed by immunohistochemistry. Tumor cells showed a heterogeneous expression; however, most of them were positive for Rictor (**b**) (original magnification, x400)

Sapanisertib, another mTORC1/2 inhibitor, has also shown promising results in advanced solid cancers in a phase I study [103] and is under clinical evaluation alone or in combination with osimertinib for the treatment of NSCLC patients (Table 2).

There are no data available regarding the efficacy of mTORC1/2 inhibitors in LAM, however, in a recent study, vistusertib has been reported to suppress epithelial-mesenchymal transition and block tumor progression in an animal model for TSC-associated neoplasms [104].

Other Types of mTOR Pathway Inhibitors

There are other types of mTOR pathway inhibitors, such as dual PI3K-mTOR inhibitors, pan-PI3K inhibitors, isoform-specific PI3K-inhibitors and Akt inhibitors [37, 50]. Many of them are under investigation in phase I and II studies.

Gedatolisib, a dual PI3K-mTOR inhibitor, is currently under investigation in combination with chemotherapies and other targeted therapies, such as the CDK4/6 inhibitor palbociclib, predominantly in patients with *PTEN* loss or activating alterations of the *PIK3CA* gene (Table 2).

Pan-PI3K inhibitors can inhibit the catalytic activity of all 4 PI3K class I isoforms (PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ), whereas isoform-specific inhibitors have been developed to target only 1 of the PI3K class I isoforms. PI3K inhibitors are widely used in phase I and II studies, some of them in selected lung cancer patients with *PTEN* loss or *PIK3CA* alterations (Table 2). Unfortunately, it has been recently reported in a biomarker-driven study (Lung-MAP) that taselisib, a PI3Kα-isoform specific inhibitor, failed to meet its primary end point in *PIK3CA*-altered SCC [105]. Based on the evolving treatment landscape and the challenging safety profile observed in the BASALT studies, buparlisib will not be further developed in lung cancer [106, 107].

Targeting of Akt prevents aberrant activation of the PI3K/Akt/mTOR signaling by modulating the downstream effects of the pathway [50]. While activating alterations of the *AKT1*, *AKT2* and *AKT3* genes are relatively rare in lung cancer (Table 1), overexpression of p-Akt occurs frequently [71, 108–110]. The potential disadvantage of these drugs is that they do not inhibit the non-Akt effectors of PI3K signaling, which can result in an increase of PI3K-dependent activation of those effectors through the release of negative feedback loops [37]. Preclinical studies revealed that Akt activation can be involved in conferring resistance to EGFR inhibitors [111, 112]. Based on this observation, a phase II study was conducted using erlotinib plus MK-2206, a highly selective inhibitor of Akt, in lung cancer patients with a predominant histology of ADC. The combination met the primary end point only in patients with EGFR wild-type carcinomas [113]. However, limited data are available about other Akt inhibitors in patients with lung cancer. Phase II studies are ongoing, 2 of them involve patients with *AKT* mutation (Table 2).

The Importance of Biomarker-Based Selection of Patients for Clinical Trials

Despite the large number of preclinical and clinical studies investigating mTOR pathway inhibitors in cancer, only a few compounds have been approved for clinical use. The lack of predictive biomarkers for treatment selection can be one of the most important barriers to the clinical translation of mTOR pathway inhibitors.

Potential biomarkers that may enable the reliable prediction of sensitivity to PI3K/Akt/mTOR inhibitors can be genetic (eg, *PIK3CA* mutation or amplification, *PTEN* loss, *AKT* mutation, and *RICTOR* amplification) and protein (eg, high p-S6 and p-Akt expression) biomarkers [50, 114].

Table 2 Selected ongoing clinical trials using PI3K/Akt/mTOR pathway inhibitors in lung neoplasms

Class	Drug	Target	In Combination With	Biomarker-based selection for PI3K/Akt/mTOR pathway inhibitor therapy	Tumor type	Phase	ClinicalTrials.gov identifier
mTORC1 inhibitors	Sirolimus	mTORC1	Alone	No	LAM	III	NCT03150914
	Sirolimus	mTORC1	Resveratrol	No	LAM	II	NCT03253913
	Sirolimus	mTORC1	Auranofin	No	NSCLC, SCLC	I/II	NCT01737502
	Sirolimus	mTORC1	Epacadostat	No	NSCLC	I	NCT03217669
	Everolimus	mTORC1	Pasireotide LAR	No	LNET	II	NCT01563354
	Everolimus	mTORC1	Ceritinib	No	NSCLC	I	NCT02321501
	ABI-009	mTORC1	Alone	No	LNET	II	NCT03670030
mTORC1/2 inhibitors	Vistusertib	mTORC1 and mTORC2	Alone	Yes (not specified)	NSCLC	II	NCT02664935
	Vistusertib	mTORC1 and mTORC2	Alone	Yes (not specified)	NSCLC	II	NCT02117167
	Vistusertib	mTORC1 and mTORC2	Durvalumab	No	NSCLC	II	NCT03334617
	Vistusertib	mTORC1 and mTORC2	Selumetinib	No	NSCLC	I/II	NCT02583542
	Vistusertib	mTORC1 and mTORC2	Navitoclax	No	SCLC	I/II	NCT03366103
	Sapanisertib	mTORC1 and mTORC2	Alone	No	NSCLC	II	NCT02417701
	Sapanisertib	mTORC1 and mTORC2	Osimertinib	No	NSCLC	I	NCT02503722
Dual pan-PI3K and mTORC1/2 inhibitors	Gedatolisib	Class I PI3K isoforms, mTORC1 and mTORC2	Paclitaxel and carboplatin	Immunohistochemical loss of PTEN, activating PI3K or inactivating PTEN gene mutations	NSCLC	I/II	NCT02920450
	Gedatolisib	Class I PI3K isoforms, mTORC1 and mTORC2	Palbociclib	PIK3CA mutation, PIK3CA copy number gain or PTEN loss	SCC	I	NCT03065062
	LY3023414	Class I PI3K isoforms, mTORC1 and mTORC2	Abemaciclib	No	NSCLC	I	NCT02079636
PI3K inhibitors	Taselisib	PI3K isoform p110α	Alone	PIK3CA mutation without RAS mutation or PTEN loss	Lung cancer	II	NCT02465060
	Alpelisib	PI3K isoform p110α	Alone	PIK3CA alterations	NSCLC	II	NCT02276027
	Serabelisib	PI3K isoform p110α	Canagliflozin	PIK3CA or KRAS mutation	Lung cancer	I/II	NCT04073680
	GSK2636771	PI3K isoform p110β	Alone	PTEN mutation or deletion	Lung cancer	II	NCT02465060
	Idelalisib	PI3K isoform p110δ	Pembrolizumab	No	NSCLC	I/II	NCT03257722
	IPI-549	PI3K isoform p110γ	Nivolumab	No	NSCLC	I	NCT02637531
	Copanlisib	Class I PI3K isoforms (p110α/β/δ/γ)	Alone	PIK3CA mutation, PTEN loss or mutation	Lung cancer	II	NCT02465060
	Copanlisib	Class I PI3K isoforms (p110α/β/δ/γ)	Nivolumab	No	NSCLC	I/II	NCT03735628
	Buparlisib	Class I PI3K isoforms (p110α/β/δ/γ)	Gefitinib	EGFR mutations, PI3KCA mutations, PTEN loss	NSCLC	I	NCT01570296
Akt inhibitors	Capivasertib	Akt1, Akt2 and Akt3	Alone	Yes (not specified)	NSCLC	II	NCT02664935
	Capivasertib	Akt1, Akt2 and Akt3	Alone	Yes (not specified)	NSCLC	II	NCT02117167
	Capivasertib	Akt1, Akt2 and Akt3	Alone	AKT mutation		II	NCT02465060

Table 2 (continued)

Class	Drug	Target	In Combination With	Biomarker-based selection for PI3K/Akt/mTOR pathway inhibitor therapy	Tumor type	Phase	ClinicalTrials.gov identifier
					Lung cancer		
	Ipatasertib	Akt1, Akt2 and Akt3	Alone	AKT mutation	Lung cancer	II	NCT02465060
	Ipatasertib	Akt1, Akt2 and Akt3	Atezolizumab	No	NSCLC	I/II	NCT03337698
	MK-2206	Akt1, Akt2 and Akt3	Erlotinib or selumetinib	No	NSCLC	II	NCT01248247

Data were accessed in October 2019 from ClinicalTrials.gov

Akt, protein kinase B; *EGFR*, epidermal growth factor receptor; *KRAS*, Kirsten rat sarcoma 2 viral oncogene homolog; *LAM*, lymphangioleiomyomatosis; LNET, lung neuroendocrine tumor; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; mTORC2, mechanistic target of rapamycin complex 2; NSCLC, non-small cell lung cancer; PI3K, phosphoinositide 3-kinase; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN, phosphatase and tensin homolog; RAS, rat sarcoma viral oncogene homolog; SCC, squamous cell lung carcinoma, SCLC, small cell lung carcinoma

Preclinical studies have supported the predictive value of *PIK3CA* mutation, but validation in clinical setting has remained controversial [105, 115–117]. In addition, a recent study has analyzed genetic alterations affecting the mTOR signaling, including *MTOR*, *TSC1*, *TSC2*, and *PIK3CA* mutations, in different solid tumors [118]. Activating genetic alterations of the mTOR pathway have been found in 45% of the patients conferring increased sensitivity to everolimus [118]. Some novel biomarker candidates, such as *AKT* mutation and *RICTOR* amplification are also under investigation [50, 119]. A recent study has revealed that *AKT1 (E17K)* mutation can be predictive for capivasertib therapy in solid tumors; however, the observed response rate was lower than with therapies targeting EGFR, anaplastic lymphoma kinase, ROS proto-oncogene 1, and v-raf murine sarcoma viral oncogene homolog B1 (BRAF) [119]. The potential predictive role of *RICTOR* amplification has also been observed in SCC and SCLC cell lines [120, 73], however, to date, clinical utility has not been proven.

A limited number of preclinical studies have also investigated the predictive role of protein biomarkers for mTOR pathway inhibitor therapy [114]. The combination of high p-Akt and high p-S6/total S6 ratio has been described as a predictor of sensitivity to everolimus [121]. Sensitivity to the PI3K inhibitor pictilisib has been associated with high baseline expression p-4E-BP1 and p-Akt [122]; furthermore, markers of hyperactive Akt signaling, including high basal p-Akt levels, have correlated with sensitivity to ipatasertib [123].

In conclusion, several inhibitors of the mTOR signaling are under clinical development; however, to date, only a few have been approved for the treatment of cancer. In order to improve the clinical translation of mTOR pathway inhibitors, we need to identify predictive biomarkers that can guide treatment decisions. In addition to biomarker-based patient selection, it would be desirable to develop more effective and less toxic dosing schedules and rational drug combinations that have the capability to overcome intrinsic and acquired resistance.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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